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PRELIMINARY EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME 1-(PHENOXYETHYL)-PIPERAZINE DERIVATIVES

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Abstract: The antioxidant properties of appropriate 1-(phenoxyethyl)-piperazine derivatives possessing significant hypotensive activity in normotensive rats were measured in human venous blood samples, *in vitro*. Ferric reducing ability of plasma (FRAP) was measured in plasma, superoxide dismutase (SOD) and catalase (CAT) activities were determined in red blood cell hemolysates. Antioxidant profile of 1-(phenoxyethyl)-piperazine derivatives was compared to Trolox and Resveratrol. The compounds were investigated in concentrations from 10^{-8} to 10^{-4} mol × L^{-1}. The most promising compounds were 1-[(4-methyl)- and 1-[(2,6-dimethyl)-phenoxyethyl]-piperazine derivatives **[1, 2]** which increased SOD activity and total antioxidant capacity (TAC). An addition of chlorine atom to methyl-phenoxy moiety decreased antioxidant properties.

Keywords: 1-(phenoxyethyl)-piperazine derivatives, antioxidant properties, FRAP, SOD, CAT

A state of increased concentration of reactive oxygen species (ROS) is a risk factor in a number of diseases such as hypercholesterolemia, atherosclerosis, hypertension, congestive heart failure, diabetes, ischemia-reperfusion and neurodegenerative diseases, as well as acute and chronic inflammatory diseases (1-3). The importance of excessive ROS formation in these diseases can be explained by the mechanism of action of excessive oxidative stress: they cause damage of proteins, lipids, and nucleic acids (4). Their role is also visible in efficiency of different antioxidative compounds, drugs, metabolites and vitamins for the maintenance of an appropriate intracellular redox potential.

Ferric reducing ability of plasma (FRAP) is a good index of total antioxidant capacity (TAC) of plasma and it includes various enzymatic and non-enzymatic antioxidant factors. Moreover, it is easy to measure (5,6). In humans, the non-enzymatic antioxidants contributing to FRAP are plasma uric acid, bilirubin, α-tocopherol and L-ascorbic acid. Plasma proteins and low molecular weight compounds containing the SH group such as glutathione (GSH) are of lower activity in FRAP assay (5). The enzymatic factors of FRAP consist in superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and GSH S-transferases (GSTs) and they play an important role in modulation of drug-induced oxidative damage. It is commonly known that heritable deficiency of the mentioned antioxidant enzymes favors ROS accumulation, and has been associated with an increased risk of vascular diseases.

Many studies have shown the deleterious effects of ROS on myocardium by both direct and indirect measurements. Various antioxidants such as SOD, CAT, GPx have been shown to prevent myocardial ischemia-reperfusion injury. The severity of this injury, the subsequent level of oxidative stress, and the interaction of antioxidants with ROS determine the effectiveness of antioxidants for cardioprotection. As an example, when ischemia-reperfusion was found to increase H_2O_2, intracellular calcium ($[Ca^{2+}]i$), malondialdehyde (MDA) content and the formation of conjugated dienes in the heart, treatment of the heart with antioxidant enzymes such as SOD plus CAT protected against these changes (7).

Recently, direct evidence, using a genetically engineered animal model, has been presented to show the importance of CAT and SOD in not only curing, but also protecting the myocardium against ischemia-reperfusion injury (7). Although the activity of CAT in the myocardium has been reported to be low, many studies have revealed its role of protecting the heart from ischemia-reperfusion (8, 9).

Due to the presence of unpaired electrons in outer orbitals, free radicals and ROS may contribute

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to damage of the antioxidant enzymes. CAT and SOD play the key role in antioxidant defence in this case. It has been reported that this enzyme system is impaired in patients with cardiovascular diseases such as hypertension or arrhythmia and that these patients are exposed to oxidant stress (10, 11).

Despite numerous adverse side effects (e.g. proatherosclerotic actions, peripheral circulatory and respiratory disturbances, hypoglycaemia during diabetic therapy), β-adrenoreceptor blocking agents (β-blockers) have been widely used in therapy, including primary hypertension, angina pectoris, myocardial infarction, heart failure and cardiac arrhythmias.

In the last ten years a new generation of cardioselective (β_1-selective adrenoreceptor) blockers, such as metoprolol, atenolol or acebutolol, as well as non-selective β-blockers with α-adrenoreceptor blocking activity (e.g. carvedilol, bucindolol) were introduced to therapy. In the case of carvedilol, beside its β-blocking and antihypertensive function, antioxidant activity *in vitro* and *in vivo* has been shown. (12, 13) In the rat brain homogenates, carvedilol protected α-tocopherol against depletion induced by ferrous ions (14). Additionally, carvedilol decreased oxidative stress in patients with heart failure and hypertension (10). Antioxidant capacities of clinically used antiarrhythmic agents e.g. mexiletine, amiodarone, propranolol and carvedilol, have been established (10, 15-20).

This paper will focus on oxidative stress and the function of antioxidant enzymes in myocardial ischemia-reperfusion.

MATERIALS AND METHODS

Chemistry

Epinephrine solutions, prepared for medical use, were obtained from Polish Pharmaceutical Corporation Polfa – Warsaw. Trolox and Resveratrol were purchased from Sigma Aldrich. Other chemicals were of analytical grade, purchased in Industrial and Commercial Company "Polish Chemical Reagents" (Poland).

The following 1-(phenoxyethyl)-piperazine derivatives (Table 1) synthesized in the Department of Technology and Biotechnology of Drugs (Medical College, Jagiellonian University, Krakow) were studied:

- 1-[(4-methyl)-phenoxyethyl]-4-(2-methoxyphenyl)-piperazine dihydrochloride **[1],**
- 1-[(2,6-dimethyl)-phenoxyethyl]-4-(2-methoxyphenyl)-piperazine dihydrochloride **[2],**
- 1-[(4-chloro-3-methyl)-phenoxyethyl]-4-(2-methoxyphenyl)-piperazine dihydrochloride **[3].**

The physicochemical and hypotensive properties as well as affinities for adrenergic receptors of the examined 1-(phenoxyethyl)-piperazine derivatives 1-3 were described formerly (21).

Study samples

Venous blood samples K_3EDTA, remaining after diagnostic tests ordered in frames of routine medical checking of big industrial enterprise employers were used in the present study. Only samples of clinically healthy subjects, 17 women and 83 men, were included in the study. Every blood sample was divided in two equal parts: the control sample and the study sample. 0.9 mL blood in the control sample was incubated with 0.1 mL physiological solution of NaCl whereas 0.9 mL of blood was incubated with 0.1 mL solution of given compound in the indicated concentrations for 15 min, in 37°C (water bath). After incubation, one mL of the whole blood was centrifuged at 1000 × g for 15 min at the room temperature. The plasma was collected and retained for determination of the FRAP. Red blood cells were washed 4 times with physiological solution of NaCl, and lysed in 4.0 mL of ice–cold double distilled water. In hemolysate samples, SOD and CAT activities were measured as described later.

The antioxidant profile of 1-(phenoxyethyl)-piperazine derivatives **[1-3]** was compared to well known antioxidants: Trolox and Resveratrol. All study compounds and antioxidant standards were investigated in concentration range from 10^{-8} to 10^{-4} mol × L^{-1}(H_2O).

Methods

TAC was assessed by method described by Benzie et al. (5). The method used for the SOD assay was first described by Misra and Fridovich (9). This method is based on the SOD ability to inhibit the epinephrine oxidation to adrenochrome. The CAT activity was measured by Aebi's method (21), which is a spectrophotometric method based on the decline of hydrogen peroxide.

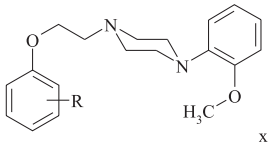
Statistical analysis

Results were expressed as relative values. TAC-FRAP in control plasma and antioxidant enzymes activities in the control hemolysates were taken as 100%. Antioxidant parameters of the investigated samples were expressed as per cent of the control samples. Differences between groups were analyzed by Anova of Kruskal-Wallis, using the STATISTICA for Windows PL software, version 6.0 (Poland).

RESULTS AND DISCUSSION

The starting point of the present study was the statement, that some 1-(phenoxyethyl)-piperazine derivatives **[1-3]** (Table 1), containing (2-methoxy)-phenylpiperazine moiety, similarly to carvedilol, are α_1- and α_2-blockers with additional β_1-adreno-receptor blocking activities (13). Compounds 1-3 inhibited ([^{3}H] prazosin) binding with K_i from 84.6 to 244.5 nM and ([^{3}H] clonidine) binding with K_i from 252.3 to 410.8 nm to cortical α_1- and α_2- adrenoreceptors, respectively. These compounds inhibited moderately also (2-[^{3}H]-CGP – 12177) binding to β_1-adrenoreceptors within μM range (K_i = 3.1 – 12.8 μM). The former studies *in vivo* showed that examined compounds **1-3** possessed a significant hypotensive activity in normotensive rats, but the effect was weaker than that of carvedilol (22). On the other hand, the toxicity of studied compounds was about half as low as that of the reference compound (22). Due to the fact that ROS play an important role in development of wide range of cardiovascular diseases we decided to determine antioxidant properties of appropriate 1-(phenoxyethyl)-4-[(2-methoxy)-phenyl]-piperazine derivatives **[1-3]** (Table 1).

Table 1. Chemical structure of the investigated compounds [**1-3**].



Compound	R
1	4-CH_3
2	2,6-CH_3
3	3-CH_3, 4-Cl

The results of this study are presented in Figures 1-3 and they show that 1-(4-methyl)- and 1-[(2,6-dimethyl)-phenoxyethyl]-piperazine derivatives increased TAC of plasma samples as well as SOD activity in red blood cells hemolysates. FRAP values increased up to 160 – 195% of original values in samples incubated with 1-[(4-methyl)-phenoxyethyl)-piperazine derivative [**1**] at concentrations of 10^{-6} – 10^{-5} mol × L^{-1}. This increase was similar to that made by Trolox and greater than an increase made by Resveratrol (p =0.05). 1-[(2,6-Dimethyl)-phenoxyethyl)-piperazine [**2**], similarly to compound **1**, increased FRAP values from 109 to 206%. The last 1-(phenoxyethyl)-piperazine derivative [**3**] reduced ferric ions only at higher concentrations and changed FRAP values as follows: enlarged FRAP by about 36% and 16% at concentration of 10^{-5} and 10^{-6} mol × L^{-1} and decreased by about 67% and 82%

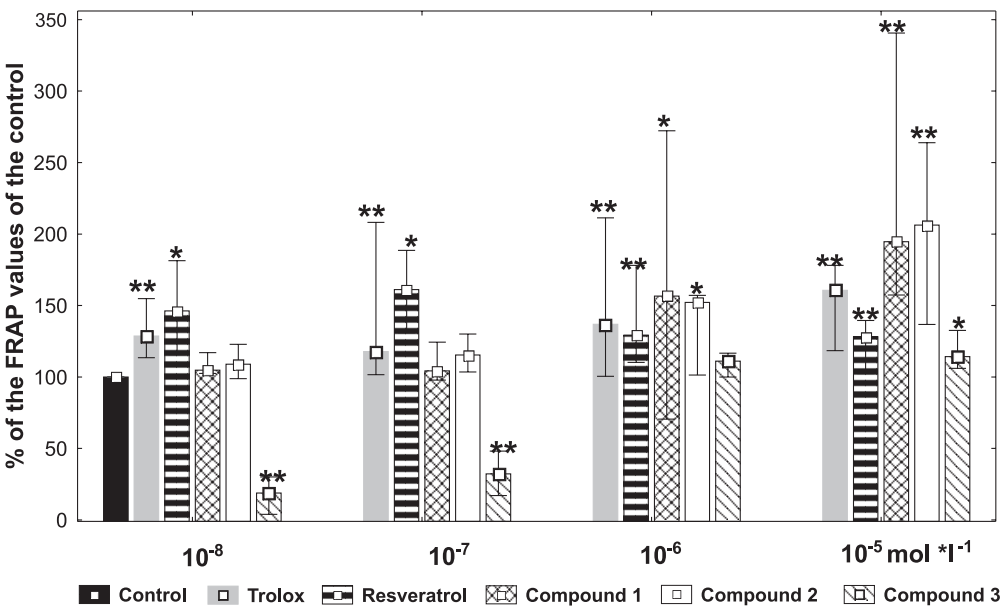


Figure 1. Effect of the studied compounds [**1-3**] on FRAP values in human plasma. * – difference between study and control samples by U test of Mann-Whitney significant at p = 0,01; or ** – at p = 0,02; rectangulars represent values of medians; whiskers show 5 – 95 percentile values.

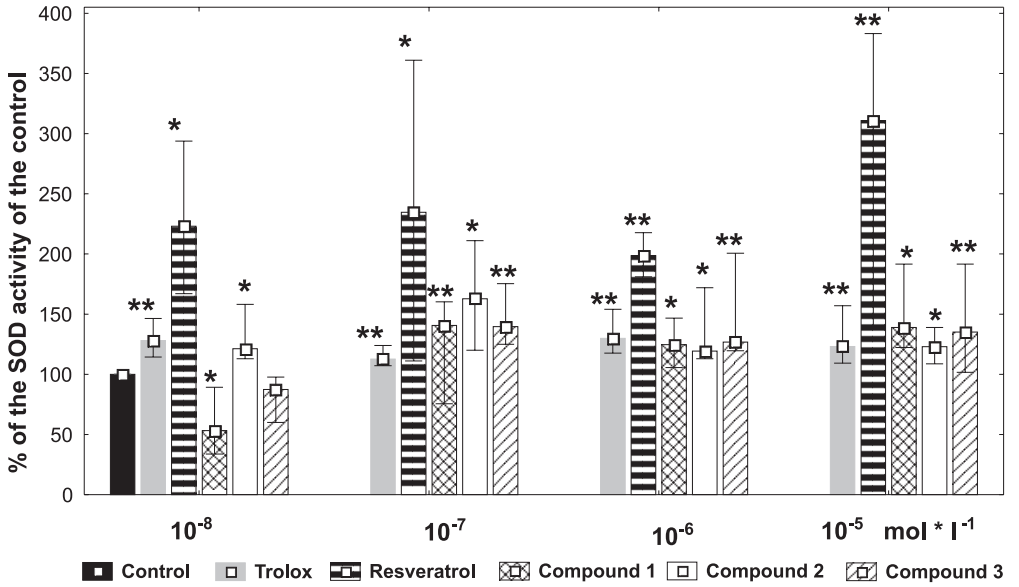


Figure 2. Effect of the studied compounds [**1-3**] on the SOD activity in human red blood cells hemolysates. * – difference between study and control samples by U test of Mann-Whitney significant at p = 0,01; or ** – at p = 0,02; rectangulars represent values of medians; whiskers show 5 – 95 percentile values.

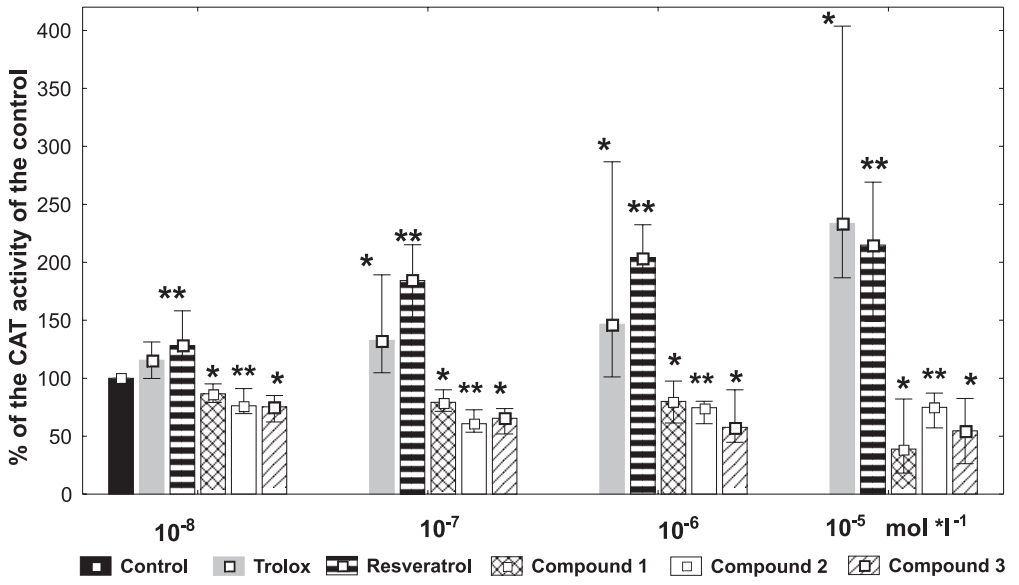


Figure 3. Effect of the studied compounds [**1-3**] on the CAT activities in human red blood cells hemolysates. * – difference between study and control samples by U test of Mann-Whitney significant at p = 0,01; or ** – at p = 0,02; rectangulars represent values of medians; whiskers show 5 – 95 percentile values.

at 10^{-7} and 10^{-8} mol × L^{-1}. The differences were statistically significant (Figure 1).

Superoxide dismutase (SOD) activity raised markedly in red blood cells hemolysates after incubation of samples with compound **1**. This compound enhanced SOD activity regardless of concentration. The highest observed increase was about 140% of original. Compounds **2** and **3** had weaker effect on this enzyme activity. It was observed that compound **2** caused an increase by about 20 – 68% of the SOD activity. The highest rise of this enzyme activity was noted at 10^{-7} mol × L^{-1}.

An addition of piperazine derivative with chlorine [**3**], to blood samples, increased SOD activity by about 40% at concentration range 10^{-7} – 10^{-5} mol × L^{-1} (Figure 2).

All tested compounds decreased CAT activity. The weaker and insignificant influence on CAT was observed in case of compound **1**. A decrease in CAT activity to 70% of original was observed (Figure 3).

The first of standard antioxidants – Trolox – increased statistically significantly every antioxidant parameter at all used concentrations. It was observed that this compound had the most effective influence on CAT activity. At concentration of 10^{-5} mol × L^{-1}, Trolox doubled CAT activity. Trolox had also considerable, but weaker, effect on CAT activity at concentration of 10^{-7}-10^{-6} mol × L^{-1}. The discussed standard antioxidant effected SOD activity regardless of concentration. There was ca. 20-30% increase of this enzyme activity. In samples incubated with Trolox, FRAP values were larger than in control samples by ca. 18-60% of the original value. The greatest rise of TAC was observed in the case of Trolox concentration of 10^{-5} mol × L^{-1}.

The second antioxidant, Resveratrol had statistically stronger antioxidant properties than Trolox. Resveratrol was an efficient activator of SOD. Doubled and tripled activity of this enzyme was observed regardless of concentration. In the case of second antioxidant enzyme, CAT, activity increased proportionally to concentration of Resveratrol from 128% at 10^{-8} mol × L^{-1}, to 215% at 10^{-5} mol × L^{-1}. Resveratrol also increased markedly TAC of plasma, but weaker than antioxidant enzymes.

The piperazine derivatives possessing 4-(methyl)- or 1-[2,6-(dimethyl)-phenoxyethyl]-moiety [**1**, **2**] were significantly stronger than the reference compounds at higher concentrations (10^{-5}-10^{-6} mol × L^{-1}). It was established that these compounds increased TAC at every concentration. The decrease of FRAP values seemed to be the result of the introduction of the second methyl or chlorine substituent in the phenyl ring.

Double substituted 1-(phenoxyethyl)-piperazine moiety significantly caused activation of SOD by compounds **2** and **3**.

1-(Phenoxyethyl)-piperazine analogues decreased CAT activity. The negative effect on CAT activity was clearly weaker after incubation of red blood cells with derivatives that had second methyl group in the molecule.

Summing up, 4-(methyl)- substituent in the molecule of 1-(phenoxyethyl)-piperazine derivatives seems to influence antioxidant properties of these compounds. Two methyl groups caused the increase of SOD activity, whereas single methyl group increased TAC. An addition of chlorine atom to appropriate (methyl)-phenoxyethyl moiety of 2-(methoxyphenyl)-piperazine [3] may decrease antioxidant properties.

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